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In Vivo Participation of Artificial Porphyrins in Electron-Transport Chains: Electrochemical and Spectroscopic Analyses of Microbial Metabolism

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Supporting Information

ABSTRACT: The effects of artificial porphyrins on the electron-transport chains of living microbes were investigated. The participation of porphyrins in the microbial electron-transport chains was demonstrated by spectroscopic and current-generation measurements. Large enhancement of the microbial current generation was accomplished by adding a cationic water-soluble manganese porphyrin as an electron mediator.

Porphyrins, such as those found in hemoglobin, myoglobin, chlorophyll, cytochrome *c* oxidase, and *c*-type cytochrome (*c*-Cyt), are widely distributed in nature. In order to understand the biological roles, in vitro studies using purified proteins have been extensively explored.[1] Artificial porphyrins have also attracted interest because of their high biocompatibility and have been applied in the field of medicine. For example, porphyrin derivatives have been used as photosensitizers in photodynamic cancer therapy,[2] and artificial oxygen carriers composed of iron porphyrin complexes have been developed as alternatives to hemoglobins.[3] In addition to these medicinal applications, numerous biomimetic studies have been performed using synthetic porphyrins for realizing artificial photosynthesis systems and oxygen reduction catalysts, among others.[4] These approaches aim at not only understanding naturally occurring porphyrins but also preparing artificial porphyrins, which are promising candidates as elements of novel energy conversion systems. However, there are few reports on porphyrins mimicking cytochrome-based respiratory electron-transport chains, whereas cytochromes, which are located in the mitochondrial inner membrane (IM), are one of the most attractive targets because of their importance in mitochondrial-generated chemical energy. One reason for the lack of progress in this area is the difficulty in evaluating the electron-transport chain within organisms.

Recently, metal-reducing bacteria of the genus *Shewanella* have attracted interest because of their unique ability to transport electrons toward extracellular matters, such as solid minerals and electrodes.[5] In *Shewanella*, the electron-transport chain has a significant content of *c*-Cyt in the outer membrane (OM), which can mediate electron transfer from the cell to attached electrodes in the course of cellular metabolism; this electron transfer results in the generation of current.[6,7] In other words, this current generation reflects the activity of electron-transport chains in living microorganisms. This strongly motivated us to investigate how artificial porphyrins would affect current generation via the electron-transport chain.

Herein, we report the participation of artificial porphyrin molecules in the electron-transport chain of living microbes for the first time. On the basis of the advantages of artificial porphyrins, such as their structural similarity to bacterial porphyrins and their variable redox potentials, we prepared artificial manganese porphyrins that can participate in in vivo respiratory electron-transport chains. By the addition of artificial manganese porphyrins to intact cells of *Shewanella loihica* PV-4,[8] the number of electrons transferred to the electrode increased, which can be explained by an increase in the number of electron-transport pathways. Because of the distinct spectral changes of manganese porphyrins that occur during redox reactions, in vivo bacterial electron transfer could be directly observed between manganese porphyrin and electron-transport chains.

To investigate the bacterial uptake of water-insoluble tetraphenylporphyrin (TPP), fluorescence microscopy measurements were carried out using fluorescent ZnTPP instead of nonfluorescent Mn^{III}TPP (Chart 1).[9] Judging from the fluorescence of individual microorganisms (Figure 1a), hydrophobic TPP was confirmed to be localized with cells.[2] Diffuse-transmission (DT) absorption spectra of a cell suspension cultivated with and without Mn^{III}TPP were measured in order to directly investigate the electronic structures of MnTPP with living microbes (Figure 1b). Three absorption bands (419, 435, and 473 nm) were observed in the Soret band region of the cells cultivated with Mn^{III}TPP. The newly formed band at 435 nm is attributable to Mn^{II}TPP,[10] while the absorption bands at 419 and 473 nm originate from ferrous *c*-Cyt and Mn^{III}TPP, respectively.[11] In the case of gram-negative bacteria such as *S. loihica* PV-4, hydrophobic TPPs are thought to be present in hydrophobic regions, such as the OMs or the lipopolysaccharide layers in the exterior

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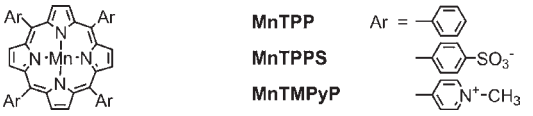
Chart 1. Chemical Structures of MnTPP, MnTPPS, and MnTMPyP



of the OMs.[2,12] Therefore, ferrous *c*-Cyt at the OMs is preferably generated by electrons supplied from NADHs continuously generated by metabolic processes via the electron-transport system consisting of quinol derivatives and *c*-Cyt at the IMs, followed by the formation of the reduced form of MnTPP mainly due to electron transfer from the outer membranous ferrous *c*-Cyt. This is supported by the cyclic voltammetry results, where the redox potential (+0.02 V vs SHE) of the *c*-Cyt of *S. loihica* PV-4 is comparable to those (−0.05 to +0.13 V vs SHE) of MnTPP observed in organic solvents.[7a,13,14]

Figure 1c shows time courses of current generation by strain PV-4 with and without treatment with water-insoluble Mn^{III}TPP. The current generated by strain PV-4 cultivated with Mn^{III}TPP increased more than 2-fold compared with cells cultivated without Mn^{III}TPP.[15] The central element of the artificial porphyrin was changed to either Fe, Co, Zn, or H_2; microbial current generation was enhanced only when the cells were cultivated with FeTPP, whose redox potential (−0.10 to +0.14 V vs SHE) is comparable to that of the *c*-Cyt of strain PV-4.[16] Because the microbial current originates from the transport of metabolic electrons toward the electrode via quinol derivatives and *c*-Cyt at the IM and OM, the increase in current generation reflects a contribution of the additional electron-transport pathways such as OM *c*-Cyts → MnTPP or FeTPP → electrodes.[17] To the best of our knowledge, this is the first report of the participation of artificial porphyrins in electron-transport chains of living microbes.

To further examine bacterial extracellular electron transfer, the water-soluble manganese(III) porphyrins tetrakis(*p*-sulfonatophenyl)porphyrin (Mn^{III}TPPS) and tetrakis(*N*-methylpyridyl)porphyrin (Mn^{III}TMPyP) were added into the medium of the reactor during microbial current generation. The immediate reduction of Mn^{III} to Mn^{II} was observed, as indicated by a color change of the supernatant for both MnTPPS and MnTMPyP (insets of Figure 2a). This reduction is ascribable to the extracellular electron transfer from *c*-Cyt in the OM of living cells to water-soluble manganese(III) porphyrins in the medium and suggests that microbial respiration requires electron transfer to molecular acceptors in addition to direct electron transfer to the electrode. From the electronic absorption spectra of the supernatant solutions, the absorption peak of MnTMPyP (450 nm) indicates the coexistence of Mn^{II} (441 nm) and Mn^{III} (463 nm) species (Figure 2a), whereas MnTPPS shows the substantial generation of Mn^{II} (433 nm) instead of Mn^{III} (467 nm) (Figure 2b).[18] The addition of anionic Mn^{III}TPPS and cationic Mn^{III}TMPyP increased the current generation of strain PV-4 by 4- and 25-fold, respectively (Figure 2c),[19] which reflects electron transfer from manganese(II) porphyrins reduced by strain PV-4 to the electrode.[20] These results suggest that MnTMPyP can efficiently function to both accept electrons from strain PV-4 and supply electrons to the electrode, whereas the electron supply is not efficient between MnTPPS and the electrode, which results in the substantial generation of the

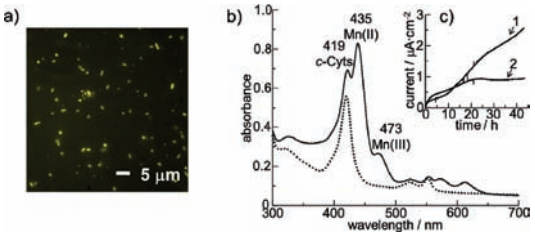


Figure 1. (a) Fluorescence microscopy image of strain PV-4 cultivated with ZnTPP. (b) DT absorption spectra of whole cells of strain PV-4 suspended in a HEPES buffer containing 10 mM lactate (DM-L) as measured in a 1.0 mm quartz cuvette. The solid and dotted lines show spectra of microbes cultivated with and without Mn^{III}TPP, respectively. The OD_{700} values were adjusted to 6.4 in the optical cuvette.[11] (c) Time profiles of current generation by strain PV-4 cultivated with (trace 1) and without (trace 2) Mn^{III}TPP.

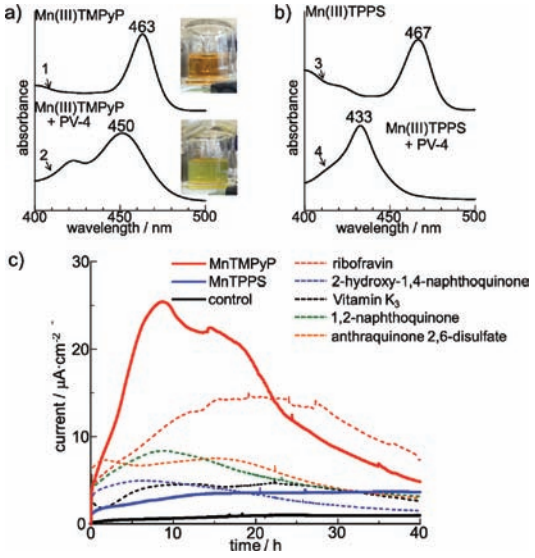


Figure 2. (a) Absorption spectra and photographs (inset) of MnTMPyP in media without (trace 1, upper) and with strain PV-4 during current generation (trace 2, bottom). (b) Absorption spectra of MnTPPS in media without (trace 3) and with strain PV-4 during current generation (trace 4). (c) Time profiles of current generation by strain PV-4 (control = black, solid) in the presence of Mn^{III}TMPyP (red, solid), Mn^{III}TPPS (blue, solid), riboflavin (red, dot), 2-hydroxy-1,4-naphthoquinone (blue, dot), Vitamin K_3 (black, dot), 1,2-naphthoquinone (green, dot), and anthraquinone 2,6-disulfate (orange, dot). The concentration of each additive in the reactor was 10 μM.

reduced Mn^{II}. In addition, MnTMPyP enhanced the current generation greater than ribofravin, naphthoquinone derivatives, and anthraquinone derivatives, which are well-known mediators that move electrons between microbes and the electrode.[21] Because only MnTMPyP is a cation, its ability to enhance current may be explained by its affinity toward the electrode surface, which is covered by microbes with a negatively charged surface (ionized phospholipids and carboxyl groups). These findings will contribute to the understanding of the requirements of efficient electron mediators between microbes and electrodes.

In summary, the effect of artificial porphyrins on microbial current generation was examined using electronic absorption spectroscopy and electrochemistry. The fluorescence from individual microbes cultivated with ZnTPP, the reduction of manganese

porphyrins by vital activity, and the current increase by redox-matching porphyrins with microbial *c*-Cyt evidenced the participation of artificial porphyrins in *c*-Cyt-based electron-transport chains of living microbes. A large enhancement of the microbial current generation was achieved by the addition of cationic water-soluble manganese porphyrins to act as electron mediators, which are expected to be useful for the fabrication of bioanode materials. Our efforts are directed toward further elucidation of this system by various approaches.

■ ASSOCIATED CONTENT

Supporting Information. Synthetic procedures for the porphyrins, the preparation for microbes, and details of spectroscopic and electrochemical measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) The ratio of the concentration of MnTPP to *c*-Cyt was estimated. See Supporting Information.

(12) In the case of gram-negative bacteria, there are hydrophobic lipopolysaccharide layers in the exterior of the OM.

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(15) See Supporting Information.

(16) To investigate the relationship between the redox potential of porphyrin and the efficiency of electron transport, current generation due to strain PV-4 cultivated with other porphyrins was measured. In the case of strain PV-4 cultivated with Fe^{III}TPP, the current increased more than twice. However, no enhancement was observed for strain PV-4 cultivated with Co^{II}TPP, Zn^{II}TPP, or H_2TPP. We concluded that MnTPP and FeTPP with redox potentials comparable to those of outer membranous *c*-Cyt could increase the number of electron-transport pathways, while CoTPP, ZnTPP, and H_2TPP with unmatched redox potentials were unable to react with *c*-Cyt.

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(19) Likewise, redox potentials are very important in the case of water-soluble porphyrins. In the case of Fe^{III}TMPyP, the current enhancement was more than 15-fold. In contrast, current generation did not increase when Co^{III}TPP or Zn^{II}TMPyP was used.

(20) After the peak, the microbial current generation decreased with a decrease in the concentration of lactate in the media. The current was partially recovered by the addition of lactate into the reactor after 40 h.[5e,7d]

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